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Lesions of a higher auditory brain area during a sensorimotor period do not impair birdsong learning

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Abstract: One important function of the nervous system is to form and retrieve memories to direct behavior. A prime example of memorization occurs in songbirds when they imitate the songs of another bird heard early in life. Although many brain areas required for song learning have been identified, their separate roles in memory formation and retrieval remain unclear. In juvenile male zebra finches, we test the role of a higher auditory cortical area in memory retrieval. The Caudal Medial Nidopallium (NCM) has previously been shown to be necessary for song memory acquisition and is thus a likely location for holding the memory of tutor song necessary for learning. We extensively exposed young birds to tutor song after which we performed large bilateral lesions in NCM using injection of ibotenic acid. We observe successful song copying in lesioned animals, comparable to song behavior in untreated control birds. Our results show that intact NCM is not required to guide vocal learning based on a previously formed song memory.

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Lesions of a higher auditory brain area during a sensorimotor period do not impair birdsong learning

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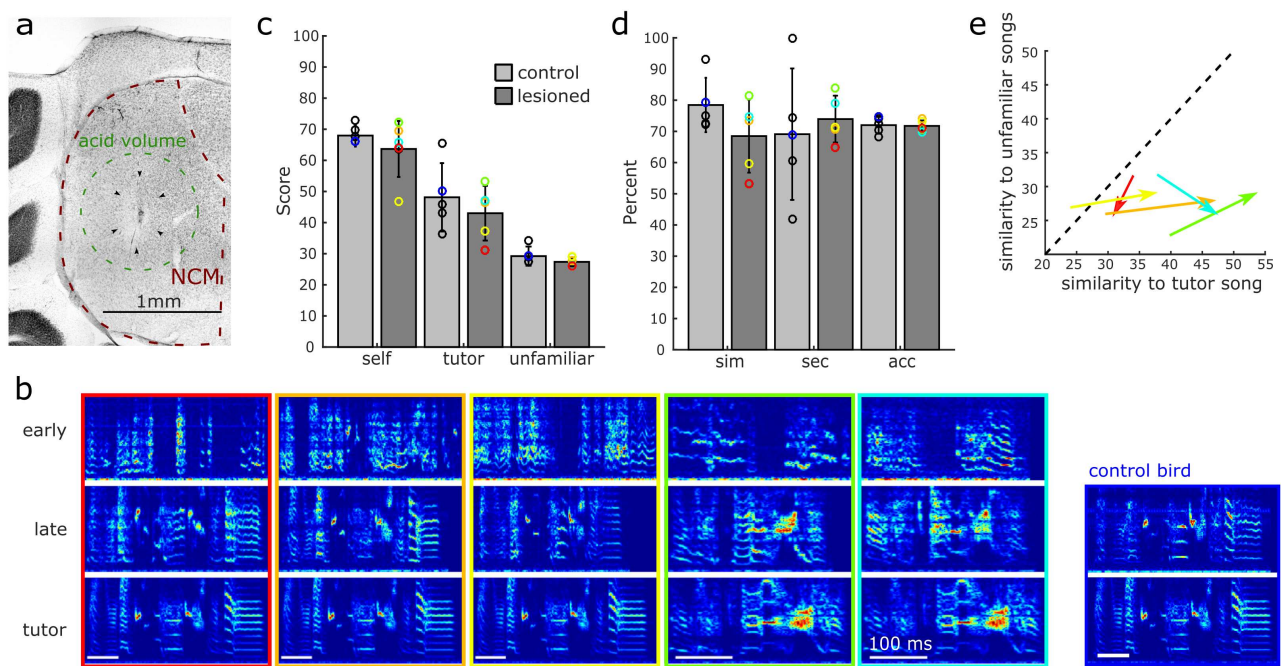
One important function of the nervous system is to form and retrieve memories to direct behavior. A prime example of memorization occurs in songbirds when they imitate the songs of another bird heard early in life. Although many brain areas required for song learning have been identified, their separate roles in memory formation and retrieval remain unclear. In juvenile male zebra finches, we test the role of a higher auditory cortical area in memory retrieval. The Caudal Medial Nidopallium (NCM) has previously been shown to be necessary for song memory acquisition and is thus a likely location for holding the memory of tutor song necessary for learning. We extensively exposed young birds to tutor song after which we performed large bilateral lesions in NCM using injection of ibotenic acid. We observe successful song copying in lesioned animals, comparable to song behavior in untreated control birds. Our results show that intact NCM is not required to guide vocal learning based on a previously formed song memory.

Objective

We investigate NCM's role in recalling of a tutor song memory. We study the effects of very large irreversible NCM lesions in about 40 dph old juvenile zebra finches that had been extensively exposed to a tutor song prior to the lesions.

Introduction

We study the organization of memory in a behavior of imitation learning based on observing and copying a conspecific's performance. We focus on birdsong development, which similarly to speech development involves perception and memorization of a sensory stimulus within a critical sensory period (Fromkin 1974^[1]) (Kuhl 2010^[2]). Juvenile zebra finches start to sing subsong (soft vocalizations comparable to the babbling of human infants) typically at an age of 30 days post hatch (dph). Gradually they transform their subsong into a good imitation of the father's song, even if separated from the latter as early as 40 dph (Immelmann K 1969^[3]) (Böhner 1990^[4]) (Funabiki 2003^[5]). A key brain area that is important for tutor song memorization is the caudal medial nidopallium (NCM). Good tutor song copying correlates with increased NCM neural activation, assessed via either immediate early genes (IEGs) (Mello 1995^[6]) (Bolhuis 2001^[7]) (Bolhuis 2000^[8]) or spiking responses (Chew 1995^[9]) (Miller Sims 2014^[10]). There is evidence that NCM holds a song memory in adult birds: tutor song recognition and recall of learned song are both impaired after irreversible lesions in NCM (Gobes 2007^[11]) (Canopoli 2014^[12]), the impairment being memory-specific because neither motor behavior nor auditory perception are significantly affected by the lesions. Evidence for a necessary role of NCM in tutor memory acquisition in juveniles comes from pharmacological manipulations: IEG suppression specifically during tutoring severely impairs tutor song learning (London 2008^[13]), paralleling the IEG requirements for memory formation in rodents (Steward 1998^[14]) (Steward 2001^[15]) (Korb 2011^[16]).



Results & Discussion

Birds were normally raised with their parents until about 40 days post hatch (dph) after which they were housed individually with no further exposure to tutor song. Hence, we make use of the fact that song learning is based on a memory of the father's song which had already been acquired before 40 dph. We investigate the role of NCM in recalling this memory to guide song learning during the sensorimotor period of birdsong development. If NCM stores the tutor song memory used as a template for learning, then the developed songs of NCM-lesioned birds should be impaired compared to the developed songs in control animals.

At 42 dph the juvenile birds received large bilateral lesions in NCM. Before the lesions, juveniles produced highly variable vocalizations (mean self-similarity score 38.0 ± 6.7 , range 26.1 to 41.7, $N=5$ birds) with little resemblance to the tutor song (mean similarity score 33.0 ± 6.4 , range 24.0 to 39.8, $N=5$ birds). At the end of the experiments, birds (76-78 dph) produced highly stereotyped song motifs (mean self-similarity score 63.7 ± 10.0 , range 46.7 to 72.4, $N=5$ birds) comparable to song stereotypy in control birds that had not received any treatment (68.0 ± 3.5 , range 63.6 to 72.8, $N=5$, $p=0.34$, t-test, Figure 1c). Most importantly, motifs of lesioned birds were good copies of their tutors' motifs: The mean similarity score in NCM-lesioned birds at 75 dph was 43.0 ± 8.8 (range 31.1 to 53.2, $N=5$ birds) comparable to the mean similarity score in age-matched controls (48.1 ± 11.0 , range 36.2 to 65.6, $N=5$ birds, $p=0.44$, t-test, example in Figure 1b, summary in Figure 1c). None of the individual similarity measures (% similarity, sequential match, and accuracy) significantly discriminated by itself between lesioned and non-lesioned birds ($p=0.17$, 0.64 and 0.86 , t-test, Figure 1d).

We tested whether the observed increase in motif similarity between NCM-lesioned birds and their tutors could be explained simply by pupils' songs becoming more similar to wild-type zebra finch song rather than specifically to the tutors' songs. We calculated similarity scores between pupils' motifs and those of 14 unfamiliar adult birds, both at the beginning and at the end of the experiment. At 43 dph, the average similarity scores with unfamiliar songs was 27.81 ± 3.86 (range 22.79 to 31.76, $N=5$ birds), and at the end of the experiments (76-78 dph) the average score was similarly low, 27.62 ± 1.40 (range 26.15 to 29.02, $N=5$ birds, $p=0.92$, t-test, Figure 1e), significantly lower than the average similarity score with tutor song ($p=0.002$, t-test). In comparison, the similarity between the controls' songs and the songs of unfamiliar adults was also significantly lower (29.2 ± 3.0 , range 26.1 to 34.2, $N=5$ birds) than the similarity between the controls' songs and tutor songs (Figure 1c, $p=0.006$, t-test). This persistence of low similarity scores with unfamiliar songs demonstrates that songs in NCM-lesioned birds developed specifically towards their tutors' songs rather than nonspecifically towards generic zebra finch songs. In summary, despite the very large lesions made, birds successfully developed a good copy of their tutors' songs.

Conclusions

The observed post-lesion convergence of pupils' songs towards their tutors' songs reveals that NCM is necessary neither for retrieving a tutor song memory nor for long-term storage of that memory. Additionally, successful song learning in NCM-lesioned birds indicates that NCM is not necessary for any of the sensorimotor functions required for song learning. Given the reported involvement of NCM in tutor song memorization (London 2008^[13]), we conclude that NCM's role in song memorization is restricted to the process of memory acquisition, after which the memory may be consolidated elsewhere in the song control system. At the very least, there must exist another song memory outside of NCM which birds can use to shape their developing songs.

Limitations

In (Canopoli 2014^[12]) it was reported that the apparent lesion size visible after Nissl staining shrinks with time. We found a similar effect of the visible lesions at 75 dph (Figure 1a) being much smaller than the injected acid volumes, which limited our ability to verify the extent of the initial lesion.

Additional Information

Methods and Supplementary Material

Please see <https://sciencematters.io/articles/201603000018>.

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Ethics Statement

All the experimental procedures were in accordance with the Veterinary Office of the Canton of Zurich.

Citations

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